

# Genetic Variants at the PDZ-Interacting Domain of the Scavenger Receptor Class B Type I Interact with Diet to Influence the Risk of Metabolic Syndrome in Obese Men and Women<sup>1-3</sup>

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## Abstract

The scaffolding protein PDZ domain containing 1 (PDZK1) regulates the HDL receptor scavenger receptor class B type I. However, the effect of *PDZK1* genetic variants on lipids and metabolic syndrome (MetS) traits remains unknown. This study evaluated the association of 3 *PDZK1* single nucleotide polymorphisms (SNP) (i33968C > T, i15371G > A, and i19738C > T) with lipids and risk of MetS and their potential interactions with diet. *PDZK1* SNP were genotyped in 1000 participants (481 men, 519 women) included in the Genetics of Lipid Lowering Drugs and Diet Network study. Lipoprotein subfractions were measured by proton NMR spectroscopy and dietary intake was estimated using a validated questionnaire. The *PDZK1*\_i33968C > T polymorphism was associated with MetS ( $P = 0.034$ ), mainly driven by the association of the minor T allele with higher plasma triglycerides ( $P = 0.004$ ) and VLDL ( $P = 0.021$ ), and lower adiponectin concentrations ( $P = 0.022$ ) than in participants homozygous for the major allele (C). We found a significant gene  $\times$  BMI  $\times$  diet interaction, in which the deleterious association of the i33968T allele with MetS was observed in obese participants with high PUFA and carbohydrate ( $P$ -values ranging from 0.004 to 0.020) intakes. Conversely, a there was a protective effect in nonobese participants with high PUFA intake ( $P < 0.05$ ). These findings suggest that *PDZK1*\_i33968C > T genetic variants may be associated with a higher risk of exhibiting MetS. This gene  $\times$  BMI  $\times$  diet interaction offers the potential to identify dietary and other lifestyle changes that may obviate the onset of MetS in individuals with a specific genetic background. J. Nutr. 139: 842–848, 2009.

## Introduction

Metabolic syndrome (MetS),<sup>10</sup> resulting from the clustering of abdominal obesity, hypertension, dyslipidemia, and hyperglycemia, is a common risk factor for atherosclerotic cardiovascular

lar diseases in populations exposed to overnutrition (1). The prevalence of MetS in the United States has been estimated at 26% and its worldwide prevalence has steadily increased over the recent years as a consequence of the global weight gain and unhealthy behaviors adopted by many populations (2).

The etiology of MetS is complex, being defined by a genetic susceptibility and environmental influences, as well as their

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<sup>10</sup> Abbreviations used: BP, blood pressure; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; DHQ, diet history questionnaire; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; HDL-C, HDL-cholesterol; IDL, intermediate-density lipoprotein; KO, knockout; LDL-C, LDL-cholesterol; LD, linkage disequilibrium; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; *PDZK1*, PDZ domain containing 1; RCT, reverse cholesterol transport; SCARB1, scavenger receptor class B type I; SNP, single nucleotide polymorphism; TG, triglyceride.

interactions (3–5). Given the number of metabolic pathways involved in MetS, many loci have been associated with individual susceptibility to MetS (3). Moreover, several twin and familial aggregation studies have also revealed a substantial genetic component for each of the MetS traits (3) and several genome-wide analyses reported suggested regions of significant linkage with the individual components of MetS at various chromosomes (6–8). However, the identification of reliable genetic markers to identify MetS risk has not been very successful, partly due to the rather incomplete knowledge of the constellation of genes implicated in this syndrome. Therefore, identification of new genes is paramount to the generation of diagnostic tools for MetS risk detection and the implementation of early prevention strategies.

The hallmarks of the dyslipidemia associated with MetS are high circulating triglyceride (TG) and low HDL-cholesterol (HDL-C) concentrations. A major player in lipoprotein metabolism is the scavenger receptor class B type I (SCARB1). Originally reported as an HDL receptor, it became evident that SCARB1 was, in fact, a multiligand receptor involved in the reverse cholesterol transport (RCT) by mediating the selective uptake of cholesteryl esters from HDL, as well as LDL and VLDL particles (9–11). In the liver, normal expression of SCARB1 protein is controlled by its adaptor PDZ domain containing 1 (PDZK1) (12,13), a 70-kDa protein composed of 4 modular PDZ-interacting domains that bind at the C terminus of SCARB1 (9). Evidence from mice (9,10,14) has shown that the loss of hepatic PDZK1 expression promotes atherosclerosis, probably due to dramatic reductions of SCARB1 activity and RCT as well as significant increases in atherogenic apoB-containing lipoproteins such as LDL and VLDL particles. Overall, current evidence supports the hypothesis that *PDZK1* is a likely candidate gene related to lipoprotein metabolism and MetS traits in humans.

*PDZK1* gene has been mapped to human chromosome 1q21, a chromosomal region that has been linked repeatedly with multiple metabolic abnormalities, such as abdominal obesity, hypertension, and MetS risk in several genome-wide linkage analyses (7,8). There are no reports, to our knowledge, examining the potential associations between single nucleotide polymorphisms (SNP) at the *PDZK1* gene with lipoprotein levels and MetS risk. Therefore, our goals in this study were to assess the association of novel polymorphisms at the *PDZK1* gene with lipoprotein levels and the MetS-related phenotypes and investigate whether *PDZK1* SNP interact with dietary factors to modulate MetS risk.

## Methods

**Subjects.** The study population consisted of 1000 participants (481 men and 519 women, age  $49 \pm 16$  y) included in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study. Participants were recruited from 3-generational pedigrees from 2 National Heart, Lung, and Blood Institute Family Heart Study field centers (Minneapolis, MN and Salt Lake City, UT) (15). The study population was all of Caucasian origin. The detailed design and methodology of the study has been described previously (16). The protocol was approved by the Institutional Review Boards at the University of Alabama, the University of Minnesota, the University of Utah, and Tufts University. Written informed consent was obtained from each participant.

**Data collection.** For GOLDN participants, clinical examinations at the baseline visit included anthropometrical and blood pressure (BP) measurements. Weight was measured with a beam balance and height with a fixed stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the umbilicus. BP was measured twice with an oscillometric device (Dinamap

Pro Series 100, GE Medical Systems) while subjects were seated and had rested for 5 min. Reported systolic and diastolic BP values were the mean of 2 measurements. Questionnaires were administered to assess demographic information and medical and medication history. Physical activity was expressed as metabolic equivalent task hours based on self-reported types and durations of activities over a period of 24 h.

The habitual dietary food intake was assessed by the Diet History Questionnaire (DHQ) developed by the National Cancer Institute (17). It consisted of 124 food items and included portion size and dietary supplement questions. The nutrient and food group database, created for analyzing the DHQ, was based on national dietary intake data from the 1994–1996 USDA's Continuing Survey of Food Intake by Individuals. These 24-h dietary recall data were used to decide which foods to include on the DHQ and what the portion sizes should be. The DHQ was administered once, when the first screening took place. Two studies have confirmed its validity (18,19).

**Laboratory methods.** Blood samples were drawn from participants after an overnight fast. Plasma was prepared and all samples stored and analyzed together after the study was complete. Glucose was measured using the method of a hexokinase-mediated reaction and total cholesterol using a cholesterol esterase cholesterol oxidase reaction on a Hitachi 911 autoanalyzer (Roche Diagnostics). The same reaction was used to measure HDL-C after precipitation of non-HDL-C with magnesium/dextran. LDL cholesterol (LDL-C) was measured by use of a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics). TG were measured by glycerol-blanked enzymatic method on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics). Plasma insulin and total adiponectin concentrations were measured using specific RIA kits (Linco Research).

Lipoprotein particle concentrations and size were measured in 995 participants by proton NMR spectroscopy (20,21). Data were obtained from the measured amplitudes of the spectroscopically distinct lipid methyl group NMR signals. We measured the concentrations of the following subfractions: large HDL (8.8–13.0 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), intermediate-density lipoprotein (IDL; 23.0–27.0 nm), small LDL (diameter 18.0–21.2 nm), large LDL (21.2–23.0 nm), large VLDL (>60 nm), medium VLDL (35.0–60.0 nm), and small VLDL (27.0–35.0 nm). The small LDL subfraction encompassed both medium small (19.8–21.2 nm) and very small (18.0–19.0 nm) particles.

**Genetic analyses.** DNA was extracted from blood samples and purified using commercial Puregene reagents (Gentra Systems) following the manufacturer's instructions. Three *PDZK1* intronic SNP (i33968C > T, rs3912316; i15371G > A, rs11576685; and i19738C > T, rs1284300) were genotyped. SNP were selected using 2 criteria: bioinformatics functional assessment and linkage disequilibrium (LD) structure. Computational analysis of *PDZK1* SNP ascribed potential functional characteristics to each variant allele. Upstream SNP analyzed by MAPPER (22) identified allele-specific transcription factor binding sites. Intronic SNP were also analyzed with MAPPER and manually checked for altered mRNA splice donor and acceptor sites and transversions affecting the poly-pyrimidine tract near splice acceptors. Minor allele frequencies of coding sequence SNP were too low to provide adequate statistical power. At the time of genotyping, the *PDZK1* reference mRNA was 64 residues shorter at the 5' end. Mapping rs11576685 to the updated *PDZK1* mRNA (accession NM\_002614.3) showed the SNP "moving" from the near promoter region to intron 1. Nonetheless, this SNP was in a putative CAAT-box weakened by the minor allele. Assessing LD structure at the *PDZK1* locus facilitated the selection of tag SNP representing different LD blocks. Of 25 blocks of tag SNP from 10 kbp upstream to 5 kbp downstream of the 36-kbp *PDZK1* gene, we selected proxy SNP from those 3 blocks that, based on our bioinformatics analysis, have a putative function. Genotyping more SNP across such a relatively small gene is not likely to add value to the phenotype-genotype association analysis. Genotyping was performed using a TaqMan assay with allele-specific probes on the ABIPrism 7900 HT Sequence Detection system (Applied Biosystems) according to routine laboratory protocols (23). The pairwise LD between SNP was estimated as the correlation coefficient (*R*) in

unrelated participants using the Helixtree software package (Golden Helix).

**Statistical analyses.** SPSS software (version 15.0) was used for statistical analyses. A logarithmic transformation was applied to measures of plasma TG, insulin, and adiponectin to normalize the distribution of the data. On the basis of the National Cholesterol Education Program-Adult Treatment Panel III guidelines (1), participants were diagnosed with MetS if they exhibited  $\geq 3$  of the following components: 1) a waist circumference  $\geq 102$  cm for men and  $\geq 88$  cm for women; 2) a plasma TG concentration of  $\geq 1.65$  mmol/L (150 mg/dL); 3) a plasma concentration of HDL-C  $< 1.04$  mmol/L (40 mg/dL) for men and  $< 1.30$  mmol/L (50 mg/dL) for women; 4) a systolic BP  $\geq 130$  mm Hg and diastolic BP  $\geq 85$  mm Hg; and 5) a fasting plasma glucose concentration  $\geq 6.10$  mmol/L (110 mg/dL). Data are presented as means  $\pm$  SD for continuous variables and as frequencies or percentages for categorical variables. Differences in mean values were assessed using ANOVA and unpaired *t* tests. Categorical variables were compared by using the Pearson chi-square or Fisher's exact tests. Potential confounding factors were age, sex, physical activity, smoking habit (current vs. never and past smokers), alcohol consumption (current vs. nondrinkers), medications (treatment for hypertension, hypercholesterolemia, diabetes, and self-reported use of hormone therapy by women), prior coronary heart disease (CHD), presence of type 2 diabetes, and family relationships. Corrections for multiple comparisons were made using the Bonferroni technique (*P*-values were multiplied by the number of analyses performed). Further adjustment for BMI was used to assess the association between *PDZK1* polymorphisms and particle subfractions. We fitted logistic regression models to estimate the OR and 95% CI of MetS across *PDZK1* genotypes stratified by dietary intakes (as dichotomous variables) and to control for the effect of covariates and total energy intake. We analyzed these data both in overall participants and also subdivided using the BMI threshold value of 30 kg/m<sup>2</sup>, based on the standard definition of obesity. As a measure of the goodness-of-fit of the models, the square of the correlation coefficient among diet components was calculated. Further adjustment for saturated fat, monounsaturated fatty acid (MUFA), PUFA, protein, and carbohydrates (as continuous variables) was used to mutually adjust intakes for each nutrient. Two-sided *P*-values  $< 0.05$  were considered significant.

## Results

Characteristics of the GOLDN participants and genotype frequencies by sex are shown (Table 1). Although BMI did not differ by sex, average weight was higher in men than in women. BP and plasma glucose and TG concentrations were higher in men, whereas HDL-C and adiponectin concentrations were higher in women. The other variables examined did not differ. For all *PDZK1* polymorphisms, there was no departure from the Hardy-Weinberg equilibrium (*P*  $> 0.05$ ). The pairwise LD in correlation coefficients of all 3 SNP is presented in Supplemental Table 1. Considering the lack of a significant *PDZK1* SNP  $\times$  gender interaction for all variables examined, data from men and women were pooled for subsequent analyses. Because of the low genotype frequencies of homozygotes for the minor alleles, we analyzed all SNP in 2 genotype categories using the dominant model to maximize the statistical power.

In the multivariate-adjusted logistic regression model, T allele carriers at the *PDZK1*\_i33968C  $>$  T SNP had a higher risk of MetS (OR 1.47; *P* = 0.034), mainly driven by the association of the minor T allele with higher TG (OR 1.50; *P* = 0.010), and a trend toward increased abdominal obesity (OR 1.22; *P* = 0.211) and hypertension (OR 1.42; *P* = 0.123) compared with CC participants (Table 2). For the *PDZK1*\_i15371G  $>$  A SNP, minor A allele carriers had more abdominal obesity than GG participants, but MetS prevalence did not differ between genotypes. We did not observe any significant association with the individual components of MetS for the *PDZK1*\_i19738C  $>$  T SNP (Table

**TABLE 1** General characteristics of the GOLDN participants<sup>1</sup>

	Men	Women
<i>n</i>	481	519
Age, y (range)	49 (18–88)	49 (18–92)
Weight, kg	90.8 $\pm$ 16.0	76.1 $\pm$ 17.5*
BMI, kg/m <sup>2</sup>	28.6 $\pm$ 4.8	28.2 $\pm$ 6.4
Waist circumference, cm	100 $\pm$ 14	93 $\pm$ 18*
Current smokers, <i>n</i> (%)	38 (8)	43 (8)
Current alcohol drinkers, <i>n</i> (%)	243 (51)	248 (48)
Physical activity, h/d	3.0 $\pm$ 1.3	2.9 $\pm$ 1.3
Systolic BP, mm Hg	119 $\pm$ 15	113 $\pm$ 18*
Diastolic BP, mm Hg	71 $\pm$ 8	66 $\pm$ 9*
Plasma glucose, mmol/L	5.83 $\pm$ 1.17	5.44 $\pm$ 0.89*
Log (plasma insulin), pmol/L	1.93 $\pm$ 1.05	1.91 $\pm$ 1.05
Total cholesterol, mmol/L	4.95 $\pm$ 0.98	5.00 $\pm$ 1.06
LDL-C, mmol/L	3.21 $\pm$ 0.78	3.11 $\pm$ 0.85†
HDL-C, mmol/L	1.06 $\pm$ 0.26	1.35 $\pm$ 0.36*
Log (TG, mmol/L)	0.24 $\pm$ 0.10	0.16 $\pm$ 0.09*
Log (plasma adiponectin), g/L	3.74 $\pm$ 0.24	3.96 $\pm$ 0.23*
MetS, <i>n</i> (%)	154 (32)	150 (29)
<i>PDZK1</i> SNP, <i>n</i> (%)		
i33968C $>$ T		
CC	354 (74)	385 (74)
CT+TT	127 (26)	134 (26)
i15371G $>$ A		
GG	449 (93)	481 (93)
AG+AA	32 (7)	38 (7)
i19738C $>$ T		
CC	401 (83)	444 (86)
CT+TT	80 (17)	75 (14)

<sup>1</sup> Values are means  $\pm$  SD unless noted otherwise. Symbols indicate different from men: \**P*  $< 0.001$ , †*P*  $< 0.05$ .

2). Given that *PDZK1*\_i33968C  $>$  T was the only SNP related to MetS, results for this SNP were examined in more detail.

Anthropometrical variables did not differ between genotype groups. However, T allele carriers had a higher log plasma TG concentration (0.24  $\pm$  0.02 mmol/L) than CC participants (vs. 0.17  $\pm$  0.01 mmol/L; *P* = 0.004). Conversely, log adiponectin was lower in T allele carriers (3.83  $\pm$  0.01  $\mu$ g/L) compared with CC participants (3.86  $\pm$  0.01  $\mu$ g/L; *P* = 0.022) (Table 3). After the Bonferroni test, T allele carriers still had higher log TG than CC participants (*P* = 0.036), whereas genotypes did not differ for log adiponectin concentrations (data not shown). Moreover, T allele carriers also tended to have higher systolic BP and higher plasma total cholesterol, glucose, and insulin concentrations than CC participants (*P* = 0.074–0.169). We also examined the effect of the *PDZK1*\_i33968C  $>$  T SNP on lipoprotein concentrations and particle size (Table 4). The only associations were found for large and medium VLDL concentrations that were higher in T allele carriers (0.41  $\pm$  0.04 and 0.61  $\pm$  0.03 g/L, respectively) than in CC participants (0.31  $\pm$  0.02 g/L, *P* = 0.021; and 0.52  $\pm$  0.02 g/L, *P* = 0.019, respectively). After multiple comparisons testing, these associations were marginally significant (*P* = 0.147 and 0.133, respectively) (data not shown).

We next examined whether the associations between *PDZK1*\_i33968C  $>$  T SNP and MetS prevalence were related to dietary habits in this population. Because there were no significant differences in the dietary intake according to genotype groups (data not shown), we investigated whether *PDZK1* gene  $\times$  diet interactions could modulate the observed association with MetS risk. We dichotomized dietary intakes according

**TABLE 2** OR for the prevalence of MetS components across PDZK1 genotype groups of the GOLDN participants<sup>1,2</sup>

	<i>n</i>	Abdominal obesity	High TG	Low HDL-C	High BP	Hyperglycemia	MetS
<i>i33968C &gt; T</i>							
CT+TT	261	1.22 (0.89–1.66)	1.50 (1.10–2.03)	0.96 (0.72–1.58)	1.42 (0.91–2.22)	1.06 (0.64–1.77)	1.47 (1.03–2.09)
CC	739	1	1	1	1	1	1
<i>P</i>		0.211	0.010	0.775	0.123	0.818	0.034
<i>i15371G &gt; A</i>							
AG+AA	70	1.84 (1.08–3.14)	1.02 (0.59–1.76)	1.45 (0.87–2.41)	1.31 (0.61–80)	1.11 (0.43–2.84)	1.46 (0.79–2.70)
GG	930	1	1	1	1	1	1
<i>P</i>		0.026	0.936	0.150	0.489	0.826	0.221
<i>i19738C &gt; T</i>							
CT+TT	155	0.83 (0.57–1.21)	0.88 (0.60–0.28)	0.79 (0.55–1.12)	0.92 (0.51–1.65)	1.09 (0.58–2.06)	0.75 (0.49–1.16)
CC	845	1	1	1	1	1	1
<i>P</i>		0.327	0.498	0.184	0.773	0.792	0.195

<sup>1</sup> Values are OR (95% CI). Models were adjusted for age, sex physical activity, smoking habit, alcohol intake status, diabetes, CHD, medications, and family relationships.

<sup>2</sup> Cut-points were based on National Cholesterol Education Program-Adult Treatment Panel III criteria (1).

to the median value for each nutrient. Our analysis showed that T allele carriers consuming diets containing low MUFA (OR 1.92;  $P = 0.015$ ), high protein (OR 1.81,  $P = 0.018$ ), or high carbohydrate (OR 1.89,  $P = 0.013$ ) intakes were associated with a higher risk of MetS than CC participants. However, no significant gene  $\times$  diet interactions were found (Supplemental Table 2). Based on previous evidence shown for other loci (24,25), we investigated whether BMI played a significant role in modifying the observed associations (Table 5). For these analyses, we dichotomized BMI above and below 30 kg/m<sup>2</sup>. Interestingly, food intake for each nutrient and total energy intake did not differ between nonobese and obese participants (data not shown). A significant gene  $\times$  BMI  $\times$  diet interaction was found ( $P = 0.004$ ) in which the deleterious association of the *i33968T* allele with MetS was observed in obese subjects with high dietary intake from PUFA ( $P = 0.015$ ), whereas a protective effect of this SNP was seen in nonobese participants ( $P = 0.024$ ). This significant gene  $\times$  BMI  $\times$  diet interaction was still observed regardless of the PUFA family investigated: (n-3) ( $P = 0.004$ ) or (n-6) ( $P = 0.006$ ). Moreover, the deleterious effect of the *i33968T* allele with MetS was observed only in obese participants with high-carbohydrate intake ( $P = 0.005$ ). Although differences were marginally significant, the association

of the minor T allele with MetS was also seen in obese participants with high-protein ( $P = 0.057$ ) and saturated fat ( $P = 0.133$ ) intakes and in nonobese participants with low-MUFA intake ( $P = 0.057$ ) (Table 5). Because PUFA intake was correlated with intakes from total fat, saturated fat, MUFA, protein, and carbohydrates ( $r = 0.759$ – $0.941$ ;  $P < 0.001$ ), models were additionally adjusted for each nutrient. Further adjustment weakened gene  $\times$  diet interactions for PUFA in nonobese participants ( $P = 0.031$ ) and for PUFA and carbohydrate components in obese participants ( $P = 0.016$  and  $P = 0.006$ , respectively), whereas the interaction with dietary protein was no longer significant ( $P = 0.060$ ) (data not shown). No significant gene  $\times$  diet interactions were found for total fat either in obese or nonobese participants.

## Discussion

In the current study, we found a significant association between *PDZK1*<sub>*i33968C > T*</sub> SNP and risk of MetS and MetS-related phenotypes. Moreover, we found a significant gene  $\times$  BMI  $\times$

**TABLE 4** Association between the *PDZK1*<sub>*i33968C > T*</sub> and lipoprotein concentrations and particle size of GOLDN participants<sup>1</sup>

	CC	CT+TT	<i>P</i> <sup>2</sup>
<i>n</i>	734	261	
HDL particle size, nm	8.86 $\pm$ 0.01	8.84 $\pm$ 0.02	0.371
LDL particle size, nm	20.82 $\pm$ 0.03	20.74 $\pm$ 0.05	0.154
VLDL particle size, nm	51.45 $\pm$ 0.29	51.52 $\pm$ 0.49	0.910
Large HDL, g/L	0.21 $\pm$ 0.04	0.20 $\pm$ 0.07	0.473
Medium HDL, g/L	0.04 $\pm$ 0.02	0.04 $\pm$ 0.03	0.429
Small HDL, g/L	0.21 $\pm$ 0.02	0.22 $\pm$ 0.03	0.381
IDL, g/L	0.05 $\pm$ 0.02	0.06 $\pm$ 0.04	0.542
Large LDL, g/L	0.55 $\pm$ 0.02	0.52 $\pm$ 0.03	0.229
Small LDL, g/L	0.58 $\pm$ 0.01	0.60 $\pm$ 0.02	0.421
Medium small LDL, g/L	0.24 $\pm$ 0.01	0.25 $\pm$ 0.01	0.600
Very small LDL, g/L	0.34 $\pm$ 0.01	0.35 $\pm$ 0.01	0.477
Large VLDL, g/L	0.31 $\pm$ 0.02	0.41 $\pm$ 0.04	0.021
Medium VLDL, g/L	0.52 $\pm$ 0.02	0.61 $\pm$ 0.03	0.019
Small VLDL, g/L	0.14 $\pm$ 0.03	0.15 $\pm$ 0.06	0.466

**TABLE 3** Association between *PDZK1*<sub>*i33968C > T*</sub> and anthropometric variables, fasting plasma lipids of the GOLDN participants<sup>1</sup>

Variable	CC	CT+TT	<i>P</i> <sup>2</sup>
<i>n</i>	739	261	
Weight, kg	82.9 $\pm$ 0.6	84.0 $\pm$ 1.0	0.387
BMI, kg/m <sup>2</sup>	28.3 $\pm$ 0.2	28.6 $\pm$ 0.3	0.398
Waist circumference, cm	96 $\pm$ 0.6	96 $\pm$ 0.9	0.752
Systolic BP, mm Hg	116 $\pm$ 0.6	117 $\pm$ 0.9	0.161
Diastolic BP, mm Hg	68 $\pm$ 0.3	69 $\pm$ 0.5	0.654
Plasma glucose, mmol/L	5.61 $\pm$ 0.03	5.72 $\pm$ 0.06	0.074
Log (plasma insulin), pmol/L	1.92 $\pm$ 0.85	1.94 $\pm$ 0.85	0.169
Total cholesterol, mmol/L	4.95 $\pm$ 0.03	5.02 $\pm$ 0.06	0.162
LDL-C, mmol/L	3.16 $\pm$ 0.03	3.19 $\pm$ 0.05	0.603
HDL-C, mmol/L	1.21 $\pm$ 0.01	1.22 $\pm$ 0.02	0.716
Log (TG) mmol/L	0.17 $\pm$ 0.01	0.24 $\pm$ 0.02	0.004
Log (plasma adiponectin), g/L	3.86 $\pm$ 0.01	3.83 $\pm$ 0.01	0.022

<sup>1</sup> Values are mean  $\pm$  SE.

<sup>2</sup> *P*-values were adjusted for age, sex, physical activity, smoking habit, alcohol intake status, diabetes, CHD, medications, and family relationships.

<sup>1</sup> Values are mean  $\pm$  SE.

<sup>2</sup> *P*-values were adjusted for age, sex, BMI, physical activity, smoking habit, alcohol intake status, diabetes, CHD, medications, and family relationships.

**TABLE 5** OR for the prevalence of MetS across PDZK1\_i33968C > T genotypes stratified by median dietary intake and BMI of GOLDN participants<sup>1</sup>

	BMI <30, n = 665				BMI ≥30, n = 335			
	CT+TT, n = 168	CC, n = 497	P <sup>2</sup>	P <sup>3</sup>	CT+TT, n = 93	CC, n = 242	P <sup>2</sup>	P <sup>3</sup>
Total fat, g/d								
<50th percentile	1.88 (0.87–4.04)	1	0.109	0.505	1.15 (0.50–2.67)	1	0.739	0.338
≥50th percentile	1.45 (0.68–3.09)	1	0.339		2.00 (0.86–4.71)	1	0.109	
Saturated fat, g/d								
<50th percentile	2.01 (0.93–4.33)	1	0.075	0.339	0.95 (0.38–2.36)	1	0.908	0.133
≥50th percentile	1.27 (0.59–2.74)	1	0.547		2.18 (0.96–4.94)	1	0.061	
MUFA, g/d								
<50th percentile	2.76 (1.27–5.98)	1	0.010	0.057	1.20 (0.52–2.78)	1	0.669	0.365
≥50th percentile	1.06 (0.50–2.27)	1	0.873		1.99 (0.84–4.68)	1	0.116	
PUFA, g/d								
<50th percentile	2.83 (1.30–6.15)	1	0.009	0.024	0.71 (0.30–1.65)	1	0.425	0.015
≥50th percentile	0.91 (0.42–1.98)	1	0.811		3.48 (1.35–9.00)	1	0.010	
PUFA (n-3), g/d								
<50th percentile	2.48 (1.14–5.38)	1	0.021	0.094	0.59 (0.25–1.44)	1	0.249	0.004
≥50th percentile	1.07 (0.50–2.28)	1	0.855		3.86 (1.48–10.10)	1	0.006	
PUFA (n-6), g/d								
<50th percentile	2.64 (1.22–5.73)	1	0.014	0.035	0.74 (0.32–1.69)	1	0.468	0.020
≥50th percentile	0.94 (0.43–2.04)	1	0.865		3.51 (1.33–9.23)	1	0.011	
Protein, g/d								
<50th percentile	1.29 (0.60–2.76)	1	0.519	0.781	0.93 (0.40–2.17)	1	0.862	0.057
≥50th percentile	1.88 (0.87–4.06)	1	0.110		2.75 (1.14–6.65)	1	0.024	
Carbohydrate, g/d								
<50th percentile	1.27 (0.59–2.73)	1	0.549	0.590	0.71 (0.31–1.66)	1	0.432	0.005
≥50th percentile	1.67 (0.77–3.63)	1	0.197		4.41 (1.66–11.69)	1	0.003	

<sup>1</sup> 50th percentiles for each dietary food component and BMI group.

<sup>2</sup> P-value between PDZK1 genotypes was adjusted for age, sex, physical activity, smoking habit, alcohol intake status, diabetes, CHD, medications, family relationships, and total energy intake (kcal).

<sup>3</sup> P-value for interaction between dietary intake and PDZK1\_i33968C > T.

diet interaction in which the deleterious association of the i33968T allele with MetS was observed in obese participants with high dietary intake from PUFA, whereas a protective effect of this SNP was seen in nonobese participants with high PUFA intake. Likewise, the deleterious association of the i33968T allele with MetS was observed in obese participants with high dietary intake from carbohydrates and marginally significant with high-saturated fat and -protein intakes. Overall, these findings suggest that dietary habits may modulate the genetic susceptibility toward developing MetS, particularly in obese participants.

It is noteworthy that this is the first study, to our knowledge, that examines in a relatively large population the effects of PDZK1\_i33968C > T SNP on lipids and MetS traits. We found that i33968T allele carriers had higher TG concentrations than CC homozygotes, supporting a key role for PDZK1 in TG metabolism. The role of PDZK1 in the metabolism of the apoB-containing lipoproteins is unclear. There is currently no evidence that this receptor is directly involved in the internalization of TG from the lipoprotein particles. However, animal studies indicated that TG concentrations were modified by PDZK1 expression (14). More specifically, mice overexpressing the receptor had lower TG concentrations, whereas PDZK1 knock-out (KO) mice had higher TG concentrations (14).

Although a number of studies from mice (9–11,14) have reported that hepatic PDZK1 plays a key role in the HDL metabolism through its interaction with SCARB1, PDZK1\_i33968C > T SNP did not modulate HDL-C concentrations in our study population. The apparently lesser role of PDZK1 in HDL me-

tabolism in humans may be due to the normal expression of SCARB1 in extrahepatic tissues and cells (e.g. macrophages) despite hepatic PDZK1/SCARB1 deficiency (9,10,26), contributing to the normal lipid transport activity. Moreover, the unaffected expression of other genes involved in the RCT pathway, such as the cholesteryl ester transfer protein (CETP), may explain the lack of association between PDZK1 genotypes and HDL-C. In this regard, Zhou et al. (27) did not find differences in HDL-C levels between PDZK1/CETP double KO mice and single CETP KO control mice, concluding that CETP expression was independent of PDZK1. Likewise, the reported uptake of cholesterol esters from LDL particles independently of SCARB1 expression (28) supports the lack of association between PDZK1 genotypes and LDL-C levels.

Importantly, i33968T allele carriers had lower adiponectin levels than CC homozygotes, suggesting a more inflammatory state that may predispose individuals toward the cluster of risk factors associated with MetS (29,30). Although hypertriglyceridemia was the only individual MetS trait significantly associated with the PDZK1\_i33968C > T SNP, we also found trends toward abdominal obesity and hypertension as well as borderline effects on glucose and insulin levels, suggesting a combination of minor effects collectively contributing to MetS. Moreover, hypoadiponectinemia has been closely related with the clinical phenotype of MetS (29), reinforcing the role of PDZK1\_i33968C > T SNP in the development of MetS. The mechanism by which this polymorphism may contribute to the observed associations is unknown. Given its location in a noncoding region, the likelihood that this SNP represents a functional mutation is low. However,

the presence of transcriptional enhancers and other regulatory elements, observed frequently in intronic regions (31), may explain our findings.

We also examined the association between the *PDZK1*<sub>i33968C</sub> > T SNP and lipoprotein concentrations and particle size. Previous studies in mice have reported increased concentrations of large HDL and VLDL particles in hepatic *PDZK1* deficiency (11,15,26,32). However, we did not find significant associations between *PDZK1* and HDL size. Normal expression of *SCARB1* in extrahepatic tissues is likely to have compensated for *PDZK1* deficiency by preventing the build-up of excess unesterified cholesterol in the plasma and limiting the size of HDL particles. As reported previously in mice (11,32), the increased concentrations of VLDL particles in *i33968T* allele carriers compared with CC homozygotes may be explained by their reduced clearance as a direct effect of an impaired uptake of VLDL by the liver as a result of *SCARB1* deficiency.

Overall, these metabolic abnormalities have been reported previously in KO mice (14,26,32) in which *PDZK1* expression was inactivated. Moreover, 2 prior studies have demonstrated that *PDZK1* deficiency promotes atherosclerosis in murine models (26,32). In this regard, Kocher et al. (26) reported that the inactivation of *PDZK1* expression in mice increased aortic atherosclerosis through the suppression of the HDL-dependent activation of endothelial nitric oxide synthase with a reduction of nitric oxide levels. Given the MetS-related phenotypes observed in *i33968 T* allele carriers, we speculate a potential downregulation of the *PDZK1* gene in those participants.

In addition to the genetic susceptibility, environmental factors such as dietary habits may contribute to MetS. Growing evidence supports the possibility that diets with low PUFA (33–36) and moderate-to-high carbohydrate contents (37,38) are associated with increased risk of MetS through mechanisms involving dyslipidemia, vascular dysfunction, and insulin resistance. Moreover, whereas epidemiological data support a protective role for PUFA (n-3) (34,39,40), the relation between (n-6) PUFA intake and cardiovascular disease risk remains still controversial (34,41). Nonetheless, PPAR $\alpha$ , a transcriptional regulator activated by dietary fatty acids, has been shown to modulate expression of *PDZK1* in humans (42).

Moreover, the potentially deleterious association of the *i33968T* allele with MetS was observed in obese participants with high dietary intake from carbohydrates. These findings suggest a synergistic relationship between genes and diet that is modulated by the BMI. It seems that obesity triggers the deleterious effect of the *PDZK1* gene leading to MetS. Overall, these gene  $\times$  diet interactions appear to support the “thrifty genotype hypothesis” (43) through which the evolutionary selection of genes that were originally beneficial for energy storage has become harmful in the setting of an environment of overnutrition. Therefore, weight loss regimens and tailored dietary recommendations targeted at reducing MetS risk might modulate the genetic expression of *PDZK1*<sub>i33968C</sub> > T SNP.

Despite the evidence, we should be cautious in the interpretation of our findings. An important limitation of the study is the fact that the data are cross-sectional and therefore the reported associations cannot presume causality. In this regard, large prospective studies with a long period of follow-up are required to clarify the directionality of these associations. Another limitation is that dietary intake was measured within a limited period of time and may not be representative of previous or habitual exposures. In addition, although we adjusted for potential confounders, residual confounding could possibly account for the findings, particularly the reported high-order (gene  $\times$  BMI  $\times$  diet) in-

teractions. The exploratory nature of the present study and the large number of comparisons make it difficult to overcome the significance of these findings and, therefore, extension of these analyses to further studies in statistically powered and carefully phenotyped population samples is clearly warranted.

In conclusion, this study conducted in a relatively large North American Caucasian population supports the hypothesis that *PDZK1*<sub>i33968C</sub> > T SNP is associated with risk of MetS, which is driven primarily by the association of the *i33968T* allele carriers with higher TG and lower adiponectin concentrations. This association was particularly evident in obese participants with high dietary intake from PUFA and carbohydrates. Interestingly, this gene  $\times$  BMI  $\times$  diet interaction offers the potential to identify dietary and other lifestyle changes that, when implemented, may obviate the onset of MetS in individuals with a specific genetic background.

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